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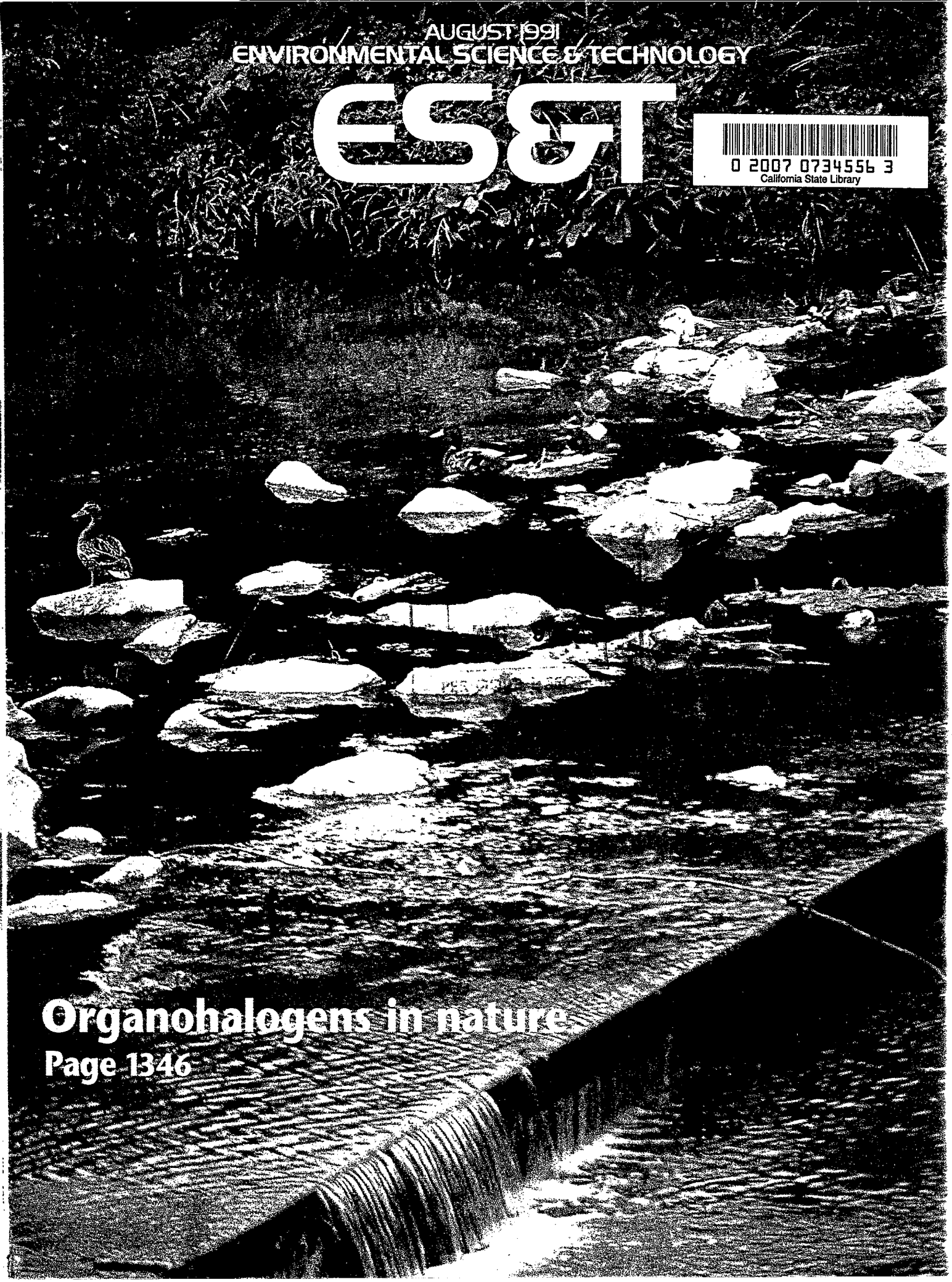

Organohalogens in nature

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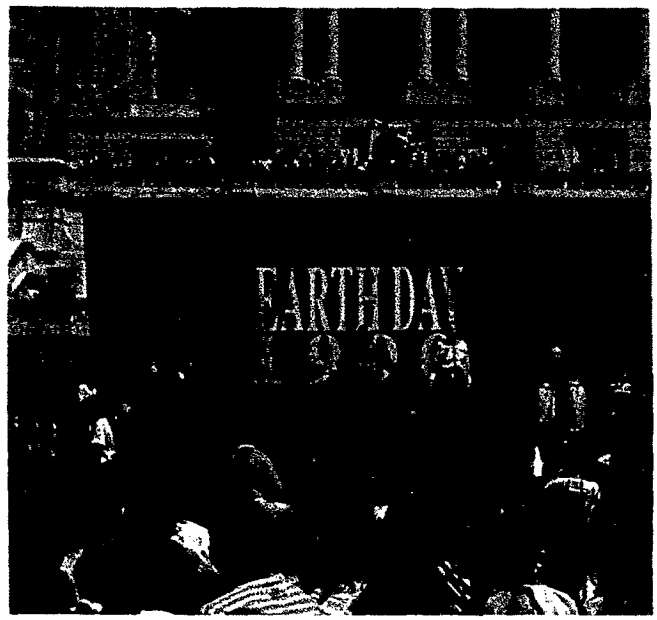

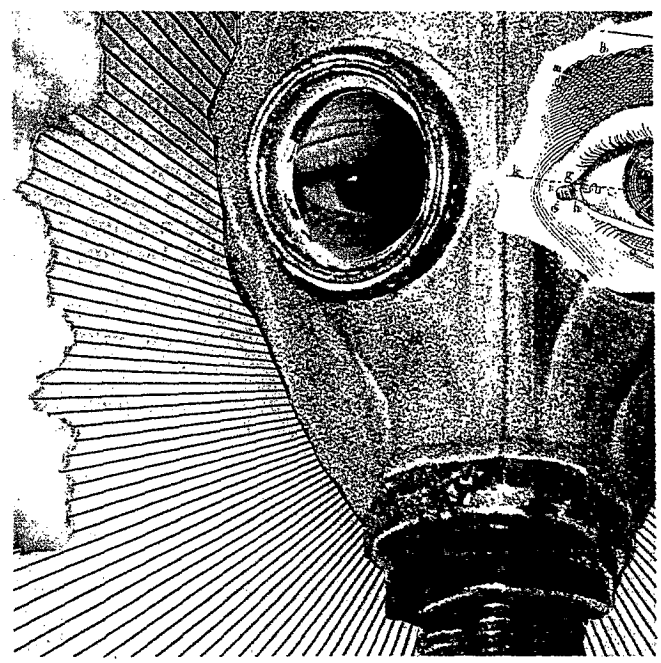

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Survey of Potable Water Supplies for *Cryptosporidium* and *Giardia*

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■ The comparative occurrence of *Cryptosporidium* and *Giardia* was evaluated in 257 water samples from 17 states in the United States. *Cryptosporidium* oocysts were detected in 55% of the surface water samples at an average concentration of 43 oocysts/100 L, while *Giardia* cysts were found in 16% of the same samples at an average concentration of 3 cysts/100 L. *Giardia* and *Cryptosporidium* were more frequently detected in samples from waters receiving sewage and agricultural discharges as opposed to pristine waters. There was no correlation between the concentration of water quality indicator bacteria and either protozoa. Both protozoa were more frequently isolated in the fall than other seasons of the year. The concentrations of both organisms were significantly correlated in all waters. *Cryptosporidium* oocysts were detected in 17% of 36 drinking water samples (0.5–1.7 oocysts/100 L) while no *Giardia* cysts were detected. The widespread occurrence of cysts and oocysts in waters used as supplies of potable water suggests that there is a risk of waterborne transmission of *Cryptosporidium* and *Giardia* infections if the water is not adequately treated.

Introduction

Cryptosporidium and *Giardia* are enteric protozoa that cause waterborne disease. Waterborne giardiasis was first recognized in the United States in 1965, and as of 1988, 106 outbreaks have been reported (*1*). *Cryptosporidium* has only recently been recognized as a cause of waterborne disease. By the 1980s *Cryptosporidium* was well documented as a cause of diarrheal illness in humans and the first waterborne outbreak was reported in 1985 (*2*).

Giardia is currently the most frequently identified agent of waterborne disease in the United States; however, in the majority of outbreaks the etiological agent has remained undetermined (*1*). Although *Cryptosporidium* has been documented in only two waterborne outbreaks, it was responsible for one of the largest outbreaks in the United States since 1920, with an estimated 13 000 individuals affected (*3*). Not only was the size of the outbreak significant, but the water underwent complete treatment including coagulation, sedimentation, rapid sand filtration, and chlorination (*4*). Water quality standards for coliforms (<1/100 mL) and turbidity (<1 NTU) were met and disinfection (1.5 mg/L chlorine) was not deficient or interrupted. However, improper or poor operational practices were identified, including poor mixing during coagulation and restarting of dirty filters without backwashing.

Many characteristics that enhance the potential for transmission through water are shared by *Cryptosporidium* and *Giardia*. Both are transmitted by the fecal–oral route, with the infected individual excreting *Cryptosporidium* oocysts or *Giardia* cysts. Animals as well as humans may serve as sources of environmental contamination and human infection. The oocyst and cyst are the environmentally stable stages and both are resistant to inactivation by drinking water disinfectants (*5*, *6*). There is no simple or routine test that can be used to evaluate the occurrence of these protozoa in water, and the bacterial indicator system used to assess microbial water quality may be inadequate for the determination of parasitological water quality (*7*).

The occurrence of the enteric protozoa in drinking water sources indicates a potential risk for waterborne disease, depending on the level of contamination and the effectiveness of the drinking water treatment. In two previous surveys, 10 and 28% of the surface waters sampled were shown to contain *Giardia* cysts at levels between 0.6 and 5/100 L (*8*, *9*). *Cryptosporidium* occysts were reported in as many as 77% of the waters examined in the western United States at concentrations of 0.1–94 oocysts/100 L (*10*). In a study limited to a single watershed, *Cryptosporidium* oocyst concentrations in water were correlated to *Giardia* cyst levels (*7*).

This survey was undertaken to gain additional information on the comparative occurrence of *Cryptosporidium* and *Giardia* in waters used for potable supplies in the United States. In particular we were interested in the occurrence of cyst and oocyst levels in pristine (more protected watersheds) and polluted waters (receiving sewage and agricultural discharges), seasonal occurrence, and association with other water quality variables.

Materials and Methods

Samples were collected from rivers, streams, lakes (or reservoirs), and springs that were used as sources of drinking water. These sites were identified with the assistance of local water authorities and utilities. Samples were categorized as polluted on the basis of the description of the watershed including public access and use, development, farms, and known point discharges from sewage treatment plants, and as pristine if there was no or little human activity, restricted public access, no agricultural activity within the watershed, or sewage treatment plant

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discharges impacting the water upstream from the sampling site. Samples were collected over the period of 1985–1988 throughout the year.

Sampling methods and procedures were adapted from those previously described (*11*). Samples were collected by passing the water through a 10-in.-long polypropylene yarn-wound cartridge filter (Micro Wynd II, AMF/Cuno, Meriden, CT, 1.0-m nominal porosity). For surface waters approximately 400-L samples were filtered. Approximately 1000-L samples were also collected from groundwater and treated drinking water by connecting the filter housing directly to a tap. Samples of backwash waters originating from sand filters at the drinking water treatment plants were also collected. Because of the high turbidity of this water only 10–40-L samples could be collected.

After the samples were collected, the filters were removed from the housing, placed in large Zip-loc plastic bags, and shipped on ice to the laboratory for processing. Most filters were processed within 2–4 days; however, in a few cases, the filters were kept at 4 °C for 7–10 days prior to processing.

Oocysts and cysts were recovered from the filter by first backwashing the filter with deionized (DI) water containing 0.1% Tween 80. The filter was cut longitudinally, teased apart, separated in halves, and washed in the eluate in a large container on a shaker at top speed (Gyrotory Shaker, Model G76, New Brunswick Sci. Co., Edison, NJ) for 10 min. The filter material was then rinsed and pressed by hand for 1 min or until clean. The washed filter material was discarded, and the eluate was combined and centrifuged at 1200*g* for 10 min. Final pellet volumes were recorded, half was resuspended into 10–20 mL of 3.7% formaldehyde for *Giardia* analysis, and the second half of the pellet was resuspended into 10–20 mL of 2.5% potassium dichromate for *Cryptosporidium* analysis. Both storage media have been shown to maintain the cyst and oocyst counts for up to 20 weeks (*13*), and oocysts have been shown to maintain viability when stored in potassium dichromate.

A volume of resuspended pellet equivalent to 100 L for surface water and 400 L for groundwater or drinking water was processed for each parasite by using procedures described by Rose et al. (*12*). After being washed, the pellet was resuspended in 10 mL of the Tween 80/sodium dodecyl sulfate solution and homogenized and one drop of antifoam agent A (Sigma, St. Louis, MO) was added. The pellet was resuspended in DI water or Tween 80/SDS solution for *Giardia* or *Cryptosporidium* processing, respectively. The mixture was sonicated for 4 min in a water bath (Branson ultrasonic cleaner 25 KHz, Shelton, CT), and layered onto sucrose [1.24 specific gravity (g/mL), 500 g of sucrose, 320 mL of DI water, and 9.7 mL of liquid phenol, diluted 4/5], for *Cryptosporidium*, and potassium citrate (1.24 g/mL, approximately 56%) was used for *Giardia* (*12*). Following centrifugation, the top and interface layers and 10 mL of the flotation medium were collected, diluted with DI water in a 1/3 ratio, pelleted, and resuspended in an appropriate volume (1–10 mL) of DI water, depending on the size of the pellet. The final concentrate was filtered through 13-mm-diameter cellulose nitrate membrane filters of 1.2-μm porosity for *Cryptosporidium* and 5.0-μm porosity for *Giardia*. Between two and six replicate membranes were needed to filter the entire sample, depending on the turbidity of the suspension.

The samples were stained with monoclonal antibodies directly on the filters while in the housing. Both direct and indirect immunofluorescence procedures were used to analyze the samples, depending on the antibodies. The development and evaluation of this methodology and antibodies that were used in this study are described in detail by Rose et al. (*13*). *Cryptosporidium* monoclonal antibodies used in this study were obtained from Dr. Charles R. Sterling, (Dept. of Veterinary Sciences, University of Arizona, Tucson, AZ) and the *Giardia* monoclonal was obtained from Meridian Diagnostics (Cincinnati, OH) (*14–16*). A second *Giardia* monoclonal antibody was provided courtesy of John L. Riggs (California State Health Department, Berkeley, CA) (*17*). Goat anti-mouse IgG or IgM(u) antibodies directly conjugated to fluorescein isothiocyanate (FITC) were obtained from Kirkegaard and Perry (Gaithersburg, MD) for an indirect fluorescence procedure.

The filters were removed from the housing and mounted in 50% glycerol phosphate buffered saline (pH 8.0), and cover slips were applied. The entire filter was examined by (200×) magnification and epifluorescence microscopy (Olympus BHTU epifluorescence microscope, New Hyde Park, NY). *Cryptosporidium* and *Giardia* were identified at 400× magnification from the following criteria: (1) bright apple-green fluorescing objects outlined by specific intense fluorescence on the outside wall of the cyst- or oocyst-like objects; (2) appropriate size and shape. For *Giardia*: oval (8–18 μm long by 5–15-μm wide). For *Cryptosporidium*: spherical (4–6 μm in diameter), characteristic folding in the oocyst wall.

The numbers of cysts and oocysts were calculated per equivalent volume examined.

Methods were evaluated by seeding known levels of oocysts and cysts into tapwater (up to 400 L) or secondary sewage effluent (up to 200 L). After the sample was processed as previously described, percent recoveries were calculated. All data for environmental samples were reported as true oocysts and cyst counts and were not adjusted for recovery efficiencies for each type of water sampled, due to the logistics in running that many seeded samples and potential for contamination of environmental samples.

Grab samples were collected in addition to filtered samples for analysis of turbidity, total coliform bacteria, fecal coliform bacteria, and in a few cases fecal streptococci bacteria and heterotrophic plate count (HPC) bacteria. Standard methods were employed using a turbidimeter, multiple tube fermentation, membrane filtration for coliforms and fecal streptococci, and spread plate techniques on plate count agar for HPC (*18*).

Each sample was defined by location, date of collection, water type, and treatment (if applicable) and categorized as polluted or pristine. Pristine samples were those coming from watersheds with little human activity, receiving no agricultural discharges or domestic sewage discharges. This information and results of each sample for the protozoa, bacteria, and turbidity were entered on a dBase III Plus program (Ashton-Tate) using an IBM personal computer. Samples were sorted by location, type of water, season, and pristine or polluted categories. Counts for cysts, oocysts, and bacterial colony forming units were transformed for analysis [log 10 (y + 1)] for all samples. Geometric averages were calculated. Pearson's correlation coefficients were developed for turbidity and each protozoan, total coliforms and the protozoa, fecal coliforms and the protozoa, and *Giardia* and *Cryptosporidium*. The SPSS-X statistical package (SPSS Inc., Chicago, IL) was used on the VAX/VMS computer at the University of Arizona Computer Center. Two-way and two-by-four-way contingency tables were set up for determining association

Table I. Surface Water Samples Collected and Analyzed for *Cryptosporidium* and *Giardia*

			Cryptosporidium		*Giardia*	
state	water type	total sample no. (pristine)[a]	no. positive (pristine)[a]	av[b] oocysts/ 100 L	no. positive (pristine)[a]	av[b] cysts/100 L
Az	river	32 (20)	14 (6)	4400	3 (2)	3.3
	lake	22 (19)	9 (7)	170	4 (4)	5.0
AR	spring	2 (2)	2 (2)	8	0	<0.25[c]
CA	river	14 (12)	6 (4)	4	1 (1)	12
	lake	7 (7)	7 (7)	6	0	<1[d]
CO	river	2 (1)	2	280	0	<1
CN	river	9 (8)	2 (2)	4	1 (0)	2.0
	lake	4 (0)	2	<1	0	<1
FL	river	3 (0)	2	5	0	<1
GA	river	2 (2)	0	<1	0	<1
HI	spring	1 (1)	1	0.25	0	<0.25
MA	lake	1 (1)	1	3	0	<1
MI	river	1 (1)	0	<1	0	<1
MO	river	3 (1)	2 (0)	8	0	<1
NY	river	5 (3)	1 (1)	2	1 (0)	2.0
	lake	6 (3)	2 (1)	1	0	<1
	spring	3 (3)	0	<0.25	0	<0.25
OR	river	7 (4)	4 (2)	3	0	<1
	lake	1 (1)	0	<1	0	<1
	spring	1 (1)	1	13	0	<0.25[c]
PA	lake	2 (0)	0	<1	0	<1
TX	river	1 (0)	1	20		
	lake	4 (3)	4 (3)	92	1 (0)	3.0
UT	river	29 (5)	23 (4)	1300	8 (1)	140
	lake	23 (0)	16	380	9	30
WA	river	3 (3)	0	<1	0	<1

[a] Number of samples in the pristine category. [b] Arithmetic averages for all positive samples. [c] Sample volume was 400 L; detection limit was <1/400 L. [d] Sample volume was 100 L; detection limit was <1/100 L.

Table II. Summary of *Cryptosporidium* and *Giardia* Occurrence in Surface Waters by Pristine versus Polluted Categories

sample category	sample no. in category	sample no. positive for *Cryptosporidium*	sample no. positive for *Giardia*	geometric av for oocysts/100 L	geometric av for cysts/100 L
surface waters	181	93	28	43	3
rivers	111[a]	57[a]	14	43	4
polluted	38	28	10	66 (29000)[b]	11 (625)[b]
pristine	59	19	4	29 (24000)[b]	0.35 (12)[b]
lakes	70[a]	39[a]	14	44	3
polluted	24	14	8	103 (7200)[b]	6.5 (156)[b]
pristine	34	18	4	9.3 (307)[b]	0.5 (7)[b]

[a] Values within a category do not add up to the total value since some samples could not be defined as to pollution category. [b] Maximum values in a single sample.

between cyst or oocyst presence and the two water categories and four seasons. Significant associations were tested by using a χ^2 analysis.

Results

Oocyst and cyst method recoveries were evaluated throughout the study by seeded trials using tapwater and activated sludge treated sewage effluent. Sample sizes ranged from 378 (tapwater) to 20 L (sewage effluent). This represented the range of the types of water we were sampling (treated potable waters to those waters highly influenced by sewage discharges). Recovery efficiencies were 29–58% for *Cryptosporidium* and 13–22% for *Giardia* and these are in the ranges previously reported in a variety of wastewaters, surface waters, and tapwaters (*9–12*).

A total of 188 surface water samples were collected from 17 states (Table I). The majority of surface water samples were taken from the western states, in particular Arizona, California, and Utah (126), while the remaining samples came from the eastern (28), northwestern (14), southern (13), and midwestern (6) states, with one sample from Hawaii. The results in Table I show the arithmetic averages for positive samples only, reflecting peak values without consideration of prevalence (percent positive) in the various water types by state for *Cryptosporidium* oocysts and *Giardia* cysts. Detection limits were 1/100 L for rivers and lakes and 1/400 L for spring waters.

Cryptosporidium oocysts were found in a greater number of samples than *Giardia* cysts and at higher concentrations. In some waters receiving wastewater discharges from sewage treatment plants and agricultural discharges (Arizona, Colorado, Texas, and Utah), high numbers of oocysts were detected. Maximum numbers were 7100, 550, 308, and 29 000/100 L (Arizona, Colorado, Texas, and Utah, respectively) for *Cryptosporidium* oocysts, and a maximum of 625 *Giardia* cysts/100 L was found in Utah waters.

Table II summarizes the occurrence of *Cryptosporidium* and *Giardia* in rivers and lakes as defined by water type and pollution category. Of the 181 river and lake water samples, 93 were categorized as pristine (receiving no sewage, agricultural, or domestic animal discharges). These sites were identified by water authorities and utilities where there was little human habitation and public access

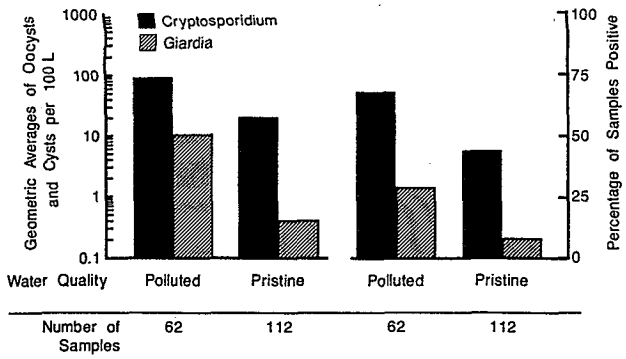

Figure 1. Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in polluted versus pristine waters.

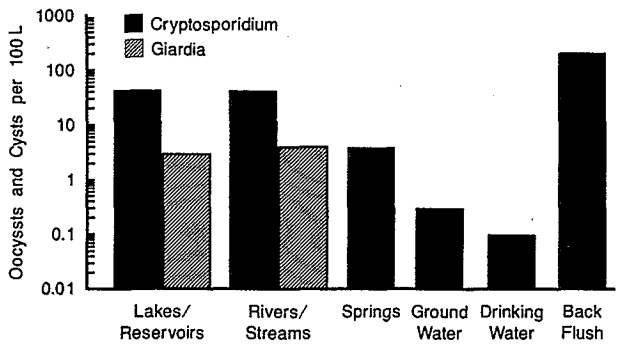

Figure 2. Geometric averages for *Cryptosporidium* oocysts and *Giardia* cysts in various water types.

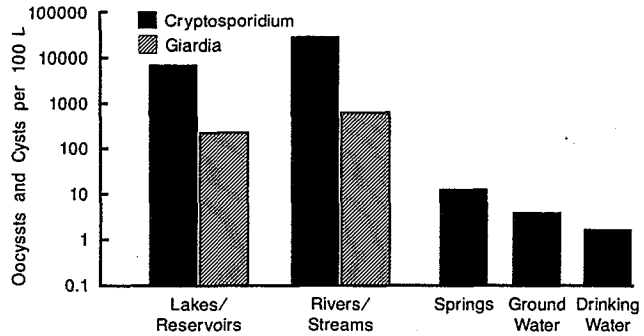

Figure 3. Peak levels of protozoan contamination in a single sample in various water types.

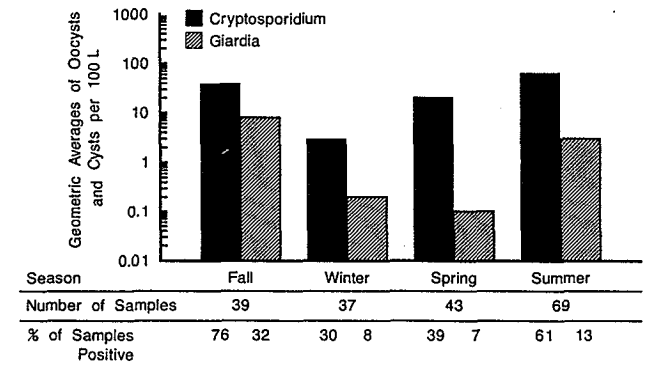

Figure 4. Seasonal occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in surface waters.

was controlled. In both river and lake samples, a greater prevalence of *Cryptosporidium* and *Giardia* was observed in the polluted category. Average oocyst and cyst levels were 11 and 13 times greater, respectively, in the polluted as opposed to pristine lake samples. In river samples, average oocysts levels were 2.2 times greater and average cyst levels were 31 times greater in the polluted category.

Seven springs were also included in the pristine category, as well as 12 groundwater samples. Twenty-six samples were undefined by pollution category and were excluded, and six groundwater samples were excluded as fluorescein tracer studies had demonstrated a plume of contamination in the well field. *Cryptosporidium* oocysts and *Giardia* cysts were detected in 39 and 7% of all pristine water samples and averaged 20 oocysts/100 L and 0.4 cysts/100 L, respectively (Figure 1). In contrast, unrpotected waters impacted by some type of discharge (sewage or agricultural) had *Cryptosporidium* in 68% of the samples with an average of 91 oocysts/100 L and *Giardia* in 29% of the samples at an average level of 10 cysts/100 L. The association between classification of a water (as polluted or pristine) and frequency of *Giardia* cyst or *Cryptosporidium* oocyst detection was evaluated with a two-way contingency table and χ^2 test of association. The χ^2 was 13.5 and 9.2 with $p < 0.005$ at one degree of freedom for *Giardia* and *Cryptosporidium*, respectively, indicating a statistically significant association between the categories of water defined in our study and the frequency of detection of these organisms.

Geometric means [log 10 (y + 1)] for all samples were calculated for the protozoa in each category of water type (Figure 2). *Giardia* cysts averaged 3 and 4/100 L in lake/reservoir and river/stream samples, respectively. *Cryptosporidium* oocyst levels were approximately 10 times higher in the surface waters (44/100 L for lake/reservoir samples and 43/100 L for river/stream samples). Spring waters had an average of 4 oocysts/100 L. Six of 36 drinking water samples were positive for oocysts and the geometric average was 0.1 oocysts/100 L. One of 18 groundwater samples was positive (4/100 L, average of 0.13 oocysts/100 L). Backwash samples from rapid sand filters produced the highest average concentration of oocysts (217/100 L).

The maximum concentrations of *Cryptosporidium* oocysts and *Giardia* cysts found in a single sample were contrasted in the various water types (Figure 3). Table II contains the maximum values in the various water pollution categories. Peak concentrations of oocysts and cysts were approximately 4 and 3 times greater, respectively, in polluted river water as compared to polluted lake water. Peak concentrations of *Cryptosporidium* oocysts were 10–100 times greater than *Giardia* cyst levels. The maximum concentrations of *Cryptosporidium* in pristine and polluted rivers were very similar, while maximum *Giardia* cyst concentrations in the same waters were over 50 times greater in polluted water as compared to pristine water. Maximum *Cryptosporidium* concentrations in springs, groundwater, and drinking water were much lower, ranging from 1.7 to 13 oocysts/100 L.

Surface water samples were sorted by season (June, July, and August as summer; September, October, and November as fall, etc.) and geometric averages were determfined for *Cryptosporidium* oocysts and *Giardia* cysts (Figure 4). The average concentrations of oocysts were the highest in the summer and fall (65 and 40/100 L, respectively). A similar pattern was also observed for average cyst concentrations (3 and 8/100 L in the summer and fall, respectively). Both oocyst and cyst concentrations were low in the winter (3 and 0.2/100 L, respectively), and in the spring only oocyst levels increased (21/100 L). The number of samples collected in the summer was 1.7–1.9 times greater than in the fall, winter, or spring. The association of oocyst and cyst occurrence with season was evaluated by using a two by four-way contingency table and the χ^2 test. A significantly greater percentage of samples (p = 0.01) were positive for cysts and oocysts in the fall. The

Table III. Geometric Averages for *Cryptosporidium*, *Giardia*, and Bacteria Concentrations by Water Type

water type	*Cryptosporidium* oocysts/100 L	*Giardia* cysts/100 L	turbidity,[a] NTU	total coliforms, CFU/100 mL	fecal coliforms,[b] CFU/100 mL	fecal streptococci,[b] CFU/100 mL	HPC,[c] CFU/mL
lakes/reservoirs	44	3	30	21	7.9	7.0	1700
rivers/streams	43	4	25	40	16	3.7	14000
springs	4	<0.25	3.5	7	ND	ND	6000
groundwater	0.3	<0.25	0.18	11	<1	ND	3900
drinking water	0.1	<0.25	0.7	<1	ND	ND	50

[a] Arithmetic averages. [b] ND, not determined. [c] Heterotrophic plate count.

Table IV. Correlation Coefficients for Turbidity, Total Coliform Bacteria, Fecal Coliform Bacteria, *Cryptosporidium*, and *Giardia* in Surface Waters

	turbidity[a]	total[b] coliforms	fecal[c] coliforms	*Cryptosporidium*[d]
Cryptosporidium	0.204	−0.032	0.026	1.0
Giardia	0.208	0.012	0.087	0.544[e]

[a] $N = 121$. [b] $N = 70$. [c] $N = 73$. [d] $N = 196$. [e] Significant at $p < 0.001$.

χ^2 was 12.3 and 12.1 for *Giardia* and *Cryptosporidium*, respectively, at three degrees of freedom with $p < 0.01$.

To determine whether season or pollution category was influencing the results of the statistical associations, the samples were sorted in respect to these variables. The samples were almost equally distributed between polluted and pristine categories for the fall (52 and 48%) winter (55 and 45%), and spring (60 and 40%) collections. In the summer, 81% of the samples fell into the pristine category; however, this apparently did not affect the trend as higher numbers of oocysts and cysts were demonstrated in the summer and fall collections. Samples from the polluted category were fairly evenly distributed among the seasons (34%, fall; 22%, winter; 24%, spring; and 19%, summer). The high percentage of pristine samples (54%) that was collected in the summer as opposed to 20, 11, and 14% that were collected in the fall, winter, and spring, respectively, did not influence the lower prevalence or numbers of oocysts and cysts found in the pristine category.

Geometric averages [log 10 (y + 1)] for both positive and negative samples for total coliforms, fecal coliforms, fecal streptococci, and HPC were calculated for samples from various water types and arithmetic averages were calculated for turbidity (Table III). Turbidity averaged 0.7 NTU in drinking water samples, with only one sample greater than 1 NTU (3.0 NTU), and was low in groundwater (0.18 NTU). Turbidity was higher in samples from springs (3.5 NTU), and averaged 25 and 30 NTU in river and lake samples, respectively. Total coliforms ranged from 7 to 40 CFU/100 mL and were not detected in drinking water. Fecal coliforms averaged 16 and 7.9 CFU/100 mL while fecal streptococci averaged 3.7 and 7 CFU/100 mL in rivers and lakes, respectively. The HPC counts averaged 50, 3900, 6000, 14000, and 17000 CFU/mL in drinking water, groundwater, springs, rivers, and lakes, respectively.

Correlation coefficients were developed for files with complete data sets in surface water samples, and no associations were observed between either protozoan and turbidity, total coliforms, or fecal coliforms. The concentrations of *Cryptosporidium* oocysts and *Giardia* cysts were significantly correlated, with an $r = 0.544$ at $p < 0.001$ (Table IV).

The surface water samples (126) from Arizona (AZ), California (CA), and Utah (UT) were sorted and evaluated separately from a category designated "all other samples" (62, surface water samples). This was done to evaluate the bias of the large number of samples collected in these states in determining trends. In both groups of samples (AZ, CA, and UT versus all other samples), similar trends were seen between the polluted and pristine categories, as previously described. A greater percentage of samples from the polluted category of waters were positive for *Cryptosporidium* oocysts and *Giardia* cysts as compared to the samples from the pristine category. In addition, the greater percentage of positive samples was found in the fall months.

Table V. Prevalence and Geometric Averages of *Cryptosporidium* Oocysts in Treated Drinking Water

	type of treatment		
	conventional[a]	direct filtration	disinfection only
sample no.	17	11	6
no. of positive samples	2	2	2
geometric av for oocysts/100 L	0.04	0.08	0.20

[a] Coagulation, sedimentation, sand filtration, and disinfection.

Table VI. *Cryptosporidium* Oocyst Concentrations, Disinfectant Residuals, and Turbidity in Treated Drinking Water[a]

source water[b]	filtration	disinfectant type	disinfectant mg/L	turbidity, NTU	oocyst/100 L
river	conventional rapid sand	chlorine	0.82	0.24	0.73
river	direct[c]	chlorine	0.9	0.5	0.57
river	dual media[b]	chlorine	1.01	0.18	0.5
river	none	chloramine	1.1	0.32	1.7
spring	none	chlorine	0.4	3.0	0.11

[a] Total coliforms were <1/100 mL for all five samples. [b] First two rivers in polluted category last two rivers in pristine category; spring influenced by a nearby river flow in polluted category. [c] Coagulant mixer inoperable. [d] No coagulants used.

The occurrences of *Cryptosporidium* oocysts in drinking water were categorized by the type of treatment the water had received. Oocysts were detected in 6 of 36 drinking water samples (400–1000 L) (Table V). In five of the positive samples, data were available on filtration, disinfection, and turbidity (Table VI). Where filtration was not used, one of the positive unfiltered samples had a turbidity (3.0 NTU) exceeding the current standard. The highest oocyst concentration detected (1.7 oocysts/100 L) also came from a water that was unfiltered. Oocysts were detected from a facility using direct filtration; however, the coagulant mixer was inoperable during sample collection. Another oocyst-positive result came from drinking water receiving direct filtration without the use of coagulants. There were no reported problems in the conventional treatment facility using rapid sand filtration in which oocysts were detected.

Table VII. Studies on the Occurrence of *Cryptosporidium* in Water Using Various Antibodies

study/antibodies used	water type	total sample no. collected	% positive	geometric av oocysts/100 L
Ongerth and Stibbs (*19*)/polyclonal	river[a]	11	100	115
rose (*10*)/IgG[b]	lake	32	75	91
	river	58	77	94
	pristine waters	6	100	2
	polluted waters	6	83	99
Stetzenbach et al. (*27*)/IgM[c]	lake	44	29	89
	river	24	29	35
this study/IgG[b] 1985–1986	pristine river	21	65	63
	polluted river	13	83	148
	pristine lake	4	50	34
	polluted lake	14	88	244
IgM[c] 1987–1988	pristine river	38	29	0.9
	polluted river	25	51	24
	pristine lake	30	47	5
	polluted lake	10	38	2

[a] Primarily pristine waters. [b] Antibody courtesy of Dr. Charles R. Sterling. [c] Antibody from Meridian Diagnostics.

Table VIII. Studies on the Occurrence of *Giardia* in Water Using Various Antibodies

study/antibodies used	water type	total sample no. collected	% positive	geometric av cysts/100 L
Ongerth and Stibbs (*9*)/polyclonal	river[a]	222	43	0.3[b]
				4
				6
Rose (*12*)/IgG[d]	pristine river	3		0.9[b]
	polluted river	8	(57)[c]	120
	polluted lake	10		35
this study/IgG[d] 1985–1986	pristine river	21	4	0.08
	polluted river	13	28	13
	pristine lake	4	0	0
	polluted lake	14	47	12
IgG[e] 1987–1988	pristine river	38	9	0.5
	polluted river	25	15	6.1
	pristine lake	30	12	1.2
	polluted lake	10	15	1.3

[a] Three separate watersheds, mostly pristine. [b] Arithmetic averages. [c] Percentage for all samples. [d] Antibody courtesy of Dr. John Riggs. [e] Antibody from Meridian Diagnostics.

Discussion

Information on the occurrence and concentrations of pathogenic microorganisms in water sources to be used as potable water supplies is critical to ensure proper treatment to protect public health. Surveys such as this one help provide information on what types of supplies may require more treatment than others and help identify seasonal and other environmental factors that influence the occurrence and concentrations of microbial pathogens. This study reports on the most extensive survey to date comparing the relative occurrence of both *Giardia* and *Cryptosporidium* in surface water supplies in the United States. This study attempted to apply the most recent advances in the detection of these protozoan parasites in water (*11–13, 19*) after a thorough evaluation of the efficiencies of these methods. Hibler reported (*8*) the most extensive study of waters (more than 4423 samples) for *Giardia* using light microscopy without the aid of antibodies. He found *Giardia* prevalence at 17–41% in lakes, rivers, and creeks. In our study, *Giardia* was detected in 16% of surface waters. Only 3% of the groundwater samples (63 wells) were positive in the Hibler study, and 3.4% of the drinking water samples (357 samples postconventional treatment) were positive for *Giardia*. In our study, no *Giardia* were detected in groundwaters or drinking waters but fewer samples were examined.

This study has demonstrated no association between the protozoa and bacterial indicators. Other surrogates appear to be needed to define water quality to enable determination of the potential risk of enteric protozoan contamination. In pristine waters indigenous animals may contribute significant numbers of oocysts and cysts to a water system on occasion. Domestic animals, particularly cattle, may also be sources of water contamination (*8, 20–23*).

Cryptosporidium oocysts were generally detected more often than *Giardia* cysts in every type of water and the concentrations of oocysts averaged 1 log greater than *Giardia* cysts. This may reflect the widespread occurrence of *Cryptosporidium* in a variety of animals. However, the levels of oocysts and cysts in all water samples were found to be significantly correlated. This supports a similar report of concurrent contamination in a single watershed (*7*).

The seasonal occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in water may be related to the seasonal occurrence of infections. Tzipori (*24*) in a recent review summarized the frequency of cryptosporidiosis in humans in Asia, the Pacific, America, Europe, and Africa. In 17 of 30 reports, the peak season was found to be in the summer or autumn, while only one study reported peak infections in the winter. Daly et al. (*25*) found that cases of giardiasis increased in midsummer and peaked in September. In a 7-year period, most of the *Giardia*-positive patients were observed in the late summer, with minimum numbers of patients in the winter (*26*).

Several previous studies have examined a variety of waters for the occurrence of *Cryptosporidum* and *Giardia* (Tables VII and VIII), (*9, 10, 12, 19, 27*). Each study employed slightly different recovery methods and different antibodies for detection. The specificity of these antibodies

to human versus animal isolates and their nonspecific binding to other organisms in water samples are variable or unknown. In addition, the method recoveries, water quality, watershed characteristics, and seasonal trends were diverse or unspecified.

Only one investigation to our knowledge has been undertaken to compare various antibodies for cyst and oocyst detection in environmental samples (*13*). To summarize briefly, the *Cryptosporidum* IgM MAb and the *Giardia* IgG (Riggs) MAb appear to be more specific for the detection of human isolates as they did not demonstrate fluorescence with one bird species (*Cryptosporidium baileyi*) or a mouse isolate (*Giardia muris*), respectively. Both *Giardia* MAb detected fewer cysts when compared to a polyclonal antibody, and the IgG MAb (Riggs) detected less than the other MAb (Meridian). In limited comparisons no differences were observed among antibodies (including those in our study) for sewage samples (*13*).

In this study and others (*10, 19, 27*) it appears that the antibodies that were less species specific for *Cryptosporidium*, including the polyclonal and the IgG, resulted in a higher percentage of samples positive and greater concentrations of oocysts than the more specific IgM antibody (Table VII). This was particularly apparent in the pristine water category. Although not as consistant or dramatic for *Giardia*, the more species-specific antibody (the IgG Riggs) did result in data of lower cyst prevalence and cyst concentrations in the pristine water category (*9, 12*) (Table VIII). The pollution categories remained distinctive regardless of the antibodies except in the case of the lake samples collected in 1987 and 1988.

These data suggest that, particularly in pristine waters, antibodies that are more specific for the species that may infect humans may result in lower counts of *Cryptosporidium* oocysts and *Giardia* cysts and may be more reflective of a human health risk. Further investigations are needed in regard to speciation, cross-infectivity, and antibody specificities for the enteric protozoa.

The occasional finding of *Cryptosporidium* oocysts in drinking water is a concern from a public health viewpoint, considering the low infectious dose of this organism (*28*) and its resistance to disinfectants (*6*). The turbidity of the treated water was below the current standard of 1 NTU in most cases, and coliform levels were in compliance. Filtration was being used in three plants, and these data as well as the large outbreaks in Carrollton (*3*) and Oxford and Swindon (*29*) demonstrate that currently we have no way of evaluating the operational parameters of a filtration plant for oocyst removal, and the adequacy of disinfection is questionable (*6*). There is no doubt that filtration can remove oocysts, as in some cases, large numbers of oocysts were detected in the backwash from filters. This could also be significant as backwash waters are often recycled through a plant to conserve water. The recycling of backwash waters was suggested as a possible contributing factor in the outbreak in England (*29*).

The consumption of nonpotable surface waters has been associated with cryptosporidiosis (*30*). Bennet et al. (*31*) have suggested that 60% of all *Giardia* infections in the United States are a result of ingesting contaminated waters (probably from both potable and nonpotable supplies, although this was not defined). The role of potable waters in the acquisition of enteric protozoan infections needs to be further evaluated. In evaluating the health significance of oocyst and cyst contamination of water, we are uncertain of viability, infectivity, and specificity of the pathogen to humans. Currently, we are unable to determine oocyst or cyst viability in environmental samples. Regardless of this limitation, the occurrence of potential pathogens in source waters may pose a significant health hazard to the exposed population, depending on a number of factors, including the level of the contamination and the degree of treatment to achieve potable water. This survey has demonstrated the widespread occurrence of *Cryptosporidium* and *Giardia* in untreated surface waters. We need to further assess pathogen distributions in water, the potential peaks of contaminants entering a drinking water supply, and the survival, transport, and infectivity of the oocyst and cyst in the environment. These data can then be used with a risk assessment approach to develop appropriate control strategies (*32*).

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Influence of the Environment on the Patina of the Statue of Liberty

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■ The copper corrosion layer, or patina, of the Statue of Liberty now shows a variation in color from one point to another that is associated with local variations in the mineralogy of the patina. It has been proposed that the color patterns are the result of attack by acid rain. To investigate this problem, a set of copper mineral phase diagrams has been prepared, which display the stability domains and solubilities as a function of the major anions (SO_4^{2-}, Cl^-, H^+, and HCO_3^-) found in rainwater. These diagrams provide the basis for a geochemical model of the patina's mineralogy. The model predicts that acid rain, at the pH levels observed in rainfall in New York City, cannot affect the mineralogy or solubility of the patina. The color patterns appear to be related to the direction of the prevailing wind, which determines where the rainwater contacts the Statue. The rainwater in turn stabilizes the sulfate copper minerals over the chloride ones. These patterns may be more prominent now than in the past because of reductions in ambient SO_2 levels in the intervening years.

I. Introduction

The restoration work on the Statue of Liberty, which was completed in 1986, was designed to eliminate the galvanic corrosion between the copper skin and the wrought iron framework. However, during the course of the work another aspect of the corrosion of the copper came to light. Baboian and Cliver (*1*) observed that there were systematic differences in the composition of the minerals of the corrison layer, or patina, at different points around the Statue of Liberty. This caused concern for two reasons. First, since antiquity the patina of a copper or bronze object has been regarded as an important esthetic factor (*2*). Second, the mineralogy of the patina affects its ability to protect the metal substrate.

Regarding this second point, Nielsen (*3*) compared samples of the present-day patina with a sample taken from the Statue of Liberty in 1905 and subsequently kept in storage. He noted that while the basic copper sulfate mineral brochantite ($Cu_4(OH)_6SO_4$) was found in samples from both periods, antlerite ($Cu_3(OH)_4SO_4$), a mineral with a higher sulfate content, was found only in the modern sample. Acid rain, a phenomenon that has appeared only in the last two or three decades, consists largely of sulfuric acid (*4*). Nielsen theorized that the acid rain was attacking the brochantite and converting it to antlerite (*2*). Since the latter is more soluble than the former, Nielsen also conjectured that acid rain may thus be increasing the rate of copper loss from the Statue. Baboian and Cliver extended this conjecture to explain the spatial pattern of color on the Statue. Analysis of old photographs indicated that the patina was uniformly green in the 1960s so that the present distribution of patina color seems to have developed over the same time period during which rain has become more acid in the Northeast United States.

However, acid rain is not the only environmental factor capable of affecting the mineralogy of the patina. Sulfur dioxide gas can react directly with the copper to produce sulfate minerals. Sea salt is another possibility, since Nielsen also found atacamite ($Cu_4(OH)_6Cl_2$) in the patina of the Statue. This basic copper chloride is often found in copper patinas in coastal areas exposed to sea-salt particles (*5*), and therefore, it is not surprising to find it on the Statue, which looks out upon the Atlantic Ocean. Thus, to understand the mineralogy of the patina it is necessary to consider the effects of the aqueous species H^+, SO_4^{2-}, and Cl^- as well as OH^-, HCO_3^-, and NO_3^-, which are also present.

A basic tool for studying the effect of environmental chemistry on corrosion layer mineralogy is the equilibrium-phase diagram, which is derived from chemical thermodynamics. These phase diagrams were originally developed by Pourbaix (*6*) to model metallic corrosion as a function of pH and redox potential, p*E*, but have subsequently been extended to a wide range of environmental variables. Since thermodynamic equilibrium is the basic requirement for constructing these diagrams, they are usually unsuitable for studying short-term kinetics.

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